IS 3198 (1965): Specification for Fodder Yeast [FAD 5: Livestock Feeds, Equipment and Systems]

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Indian Standard

SPECIFICATION FOR FODDER YEAST

UDC 636·085 : 664·642

INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 1

Price Rs 5·00 Gr 7

December 1965

Indian Standard

SPECIFICATION FOR FODDER YEAST

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AMENDMENT NO. 1 AUGUST 1996
TO
IS 3198 : 1965 SPECIFICATION FOR FODDER YEAST

(*Page* 6, *clause* 5.2, *line* 2) — Substitute 'IS 1070 : 1992*' *for* 'IS 1070 - 1960*.'

(*Page* 6, *foot-note with* ' * ' *mark*) — Substitute 'Reagent grade water (*third revision*)' for the existing title.

(*Page* 8, *Appendix* C, *clause* C-2.3) — Substitute 'IS 266 : 1993*' *for* 'IS : 266 - 1961*'

(*Page* 8, *foot-note with* ' * ' *mark*) — Substitute '(*third revision*)' *for* '(*revised*)'.

(FAD 5)

Reprography Unit, BIS, New Delhi, India

Indian Standard

SPECIFICATION FOR FODDER YEAST

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 30 July 1965, after the draft finalized by the Edible Starches, Confectionery and Cereal Products Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Fodder yeast is used for supplementing animal feeds with protein and vitamins of B Complex group. It is manufactured from surplus, cheap or waste carbohydrate or sugar sources, such as sugar cane juice, molasses, fruit juice, citrus waste, protein waste liquors and mashed grains and also from hydrolysed wood and sulphite liquor from the paper industry by growing organisms, such as *Saccharomyces*, *Torula* and *Candida* yeasts. The yeast thus obtained is separated, washed and suitably dried to kill the cells and obtain inactive fodder yeast.

0.3 This standard contains clause **3.1** which calls for an agreement between the purchaser and the vendor at the time of placing order.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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1. SCOPE

1.1 This standard prescribes the requirements and the methods of test for fodder yeast as livestock and poultry feed.

2. REQUIREMENTS

2.1 The material shall be in the form of powder, granules, pellets, or flakes. It shall be of uniform creamy white to yellowish brown colour and shall have the characteristic taste and odour of good quality yeast,

* Rules for rounding off numerical values (*revised*).

free from any unpleasant, musty or putrid smell. It shall be dry, free from lumps, visible mould growth and insect infestation. It shall be free from any extraneous matter, added colour and any deleterious substances.

NOTE — The appearance, taste and odour shall be determined by organoleptic tests.

2.2 The material shall also comply with the following requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR FODDER YEAST

SL No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST (REF TO APPENDIX)
(1)	(2)	(3)	(4)
i)	Moisture, percent by weight, *Max*	8·0	A
ii)	Total ash (on dry basis), percent by weight, *Max*	9·0	B
iii)	Crude protein (N × 6·25) (on dry basis), percent by weight, *Min*	45·0	C
iv)	Crude fibre (on dry basis), percent by weight, *Max*	1·0	D
v)	Thiamine, milligram in 100 g, *Min*	2·0	E
vi)	Riboflavin, milligram in 100 g, *Min*	3·0	F
vii)	Nicotinic acid, milligram in 100 g, *Min*	30·0	G
viii)	Pantothenic acid, milligram in 100 g, *Min*	10·0	H
ix)	Viability	To pass the test	J

3. PACKING AND MARKING

3.1 Packing — The material shall be packed in moisture-proof containers as agreed to between the purchaser and the vendor.

3.2 Marking — Each container shall be suitably marked to give the following particulars:

a) Name of the material and brand, if any;
b) Name and address of the manufacturer;
c) Date of manufacture;
d) Batch or code number; and
e) Net weight.

3.2.1 Each container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act, and the Rules and Regulations made thereunder. Presence of this mark on products covered by an Indian Standard

conveys the assurance that they have been produced to comply with the requirements of that standard, under a well-defined system of inspection, testing and quality control during production. This system, which is devised and supervised by ISI and operated by the producer, has the further safeguard that the products as actually marketed are continuously checked by ISI for conformity to the standard. Details of conditions, under which a licence for the use of the ISI Certification Mark may be granted to the manufacturers or processors, may be obtained from the Indian Standards Institution.

4. SAMPLING

4.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in Appendix K.

5. TESTS

5.1 Tests shall be carried out as prescribed in **2.1** and col 4 of Table 1.

5.2 Unless specified otherwise, pure chemicals shall be employed in tests, and distilled water (*see* IS : 1070-1960*) shall be used where the use of water as a reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

APPENDIX A

[*Table* 1, *Item* (i)]

DETERMINATION OF MOISTURE

A-1. PROCEDURE

A-1.1 Weigh accurately about 5 g of the powdered material in a moisture dish made of porcelain, silica or platinum, previously dried in an air-oven and weighed. Shake the dish until the contents are evenly distributed. Place the dish in an air-oven maintained at 105° ±2°C, and dry for at least two hours. Cool in a desiccator and weigh. Heat again at 105° ±2°C in the air-oven for 30 minutes, cool the dish in the desiccator and weigh. Repeat the process of heating for 30 minutes, cooling and weighing until the difference between two successive weighings is less than one milligram. Note the lowest weight.

NOTE — Preserve this dried material in the desiccator for the determination of crude fibre (**D-2.1**).

* Specification for water, distilled quality (*revised*).

A-2. CALCULATION

A-2.1 Moisture, percent by weight $= \frac{100\ (W_1 - W_2)}{W_1 - W}$

where

W_1 = weight in g of the dish with the material before drying,

W_2 = weight in g of the dish with the dried material, and

W = weight in g of the empty dish.

APPENDIX B

[*Table* 1, *Item* (ii)]

DETERMINATION OF TOTAL ASH

B-1. PROCEDURE

B-1.1 Weigh accurately about 10 g of the material in a tared porcelain, silica or platinum dish. Ignite with the flame of a suitable burner for about one hour. Complete the ignition by keeping in a muffle furnace at 600°±20°C, until grey ash results. Cool in a desiccator and weigh. Ignite the dish again in the muffle furnace for 30 minutes, cool and weigh. Repeat this process of igniting in a muffle furnace, cooling and weighing until the difference in weight between the two successive weighings is less than one milligram. Note the lowest weight.

B-2. CALCULATION

B-2.1 Total ash (on dry basis), percent by weight $= \frac{10\ 000\ (W_2 - W)}{(100 - M)\ (W_1 - W)}$

where

W_2 = lowest weight in g of the dish with ash,

W = weight in g of empty dish,

M = moisture, percent by weight (**A-2.1**), and

W_1 = weight in g of the dish with the material taken for test.

APPENDIX C

[*Table* 1, *Item* (iii)]

DETERMINATION OF CRUDE PROTEIN

C-1. APPARATUS

C-1.1 Kjeldahl Flask — 500 ml capacity.

C-1.2 Distillation Assembly — The assembly consists of a round-bottom flask of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube. The other end of the bulb tube is connected to the condenser which is attached by means of the rubber tube to a dip tube which dips into a known quantity of standard sulphuric acid contained in a beaker of 250 ml capacity.

C-2. REAGENTS

C-2.1 Potassium Sulphate or Anhydrous Sodium Sulphate

C-2.2 Copper Sulphate

C-2.3 Concentrated Sulphuric Acid — sp gr 1·84 (*see* IS : 266-1961*).

C-2.4 Sodium Hydroxide Solution — Dissolve about 225 g of sodium hydroxide in 500 ml of water.

C-2.5 Standard Sulphuric Acid — 0·1 N.

C-2.6 Standard Sodium Hydroxide — 0·1 N.

C-2.7 Methyl Red Indicator Solution — Dissolve one gram of methyl red in 200 ml of rectified spirit (95 percent by volume).

C-3. PROCEDURE

C-3.1 Transfer carefully 2 g of the material, accurately weighed, to the Kjeldahl flask, taking precaution to see that the particles of the material do not stick on to the neck of the flask. Add about 10 g of potassium sulphate or anhydrous sodium sulphate, about 0·2 to 0·3 g of copper sulphate and 20 ml of concentrated sulphuric acid. Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase the heat until acid boils vigorously and digest for 30 minutes after the mixture becomes clear and pale green or colourless. Cool the contents of the flask.

C-3.2 Transfer the contents quantitatively to the round-bottom flask, with water, the total quantity of water used being about 200 ml. Add

* Specification for sulphuric acid (*revised*).

with shaking a few pieces of pumice stone to prevent bumping. Add about 50 ml of sodium hydroxide solution (which is sufficient to make the solution alkaline) carefully through the sides of the flask, so that it does not mix at once with the acid solution, but forms a layer below the acid layer. Assemble the apparatus taking care that the dip tube extends below the surface of standard sulphuric acid contained in the beaker. Mix the contents of the flask by shaking and distil until ammonia has passed over into the standard sulphuric acid. Shut off the burner and immediately detach the flask from the condenser. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker. When all washings have drained into the beaker, add two to three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution.

C-3.3 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

C-4. CALCULATION

C-4.1 Total crude protein (on dry basis), percent by weight $= \frac{875\ (B-A)\ N}{(100-M)\ W}$

where

B = volume in ml of the standard sodium hydroxide solution used to neutralize the acid in the blank determination;

A = volume in ml of the standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material;

N = normality of the standard sodium hydroxide solution;

M = moisture, percent by weight (**A-2.1**); and

W = weight in g of material taken for test.

APPENDIX D

[*Table* 1, *Item* (iv)]

DETERMINATION OF CRUDE FIBRE

D-1. REAGENTS

D-1.1 Dilute Sulphuric Acid — 1·25 percent (*w*/*v*), accurately prepared.

D-1.2 Dilute Sodium Hydroxide Solution — 1·25 percent (*w*/*v*), accurately prepared.

D-1.3 Petroleum Ether — of boiling point 40° to 60°C.

D-2. PROCEDURE

D-2.1 Weigh accurately about 2 g of dried material preserved after determining moisture (**A-1.1**) and extract for about 8 hours with petroleum ether using a Soxhlet or other suitable extractor. Transfer the fat-free dry residue to a one-litre conical flask. Take 200 ml of dilute sulphuric acid in a beaker and bring to the boil. Transfer the whole of the boiling acid to the flask containing the fat-free material and immediately connect the flask with a water-cooled reflux condenser and heat, so that the contents of the flask begin to boil within a minute. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and in contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter the contents through fine linen (about 18 threads to the centimetre) held in a funnel and wash with boiling water until the washings are no longer acidic to litmus. Bring to the boil some quantity of the sodium hydroxide solution under a reflux condenser. Wash the residue on the linen into the flask with 200 ml of the boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with the boiling water and transfer to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly, first with hot water and then with about 15 ml of 95 percent (by volume) ethyl alcohol. Dry the Gooch crucible and contents at $105° \pm 1°C$ in an air-oven to constant weight. Cool and weigh. Incinerate the contents of the Gooch crucible at $600° \pm 20°C$ in a muffle furnace, until all the carbonaceous matter is burnt. Cool the crucible containing the ash in a desiccator and weigh.

D-3. CALCULATION

D-3.1 Crude fibre (on dry basis), percent by weight $= \frac{100\ (W_1 - W_2)}{W}$

where

W_1 = weight in g of Gooch crucible and contents before ashing,

W_2 = weight in g of Gooch crucible and ash, and

W = weight in g of dry material taken.

APPENDIX E

[*Table* 1, *Item* (v)]

DETERMINATION OF THIAMINE

E-1. APPARATUS

E-1.1 Fluorometer — any instrument suitable for accurately measuring fluorescence. In all cases, operating procedures, arrangements of filters and accessory equipment should be as recommended by manufacturer.

E-1.2 Water-Bath or Steam-Bath — maintained at 100°C.

E-1.3 Incubator or Water-Bath — maintained at 37° to 40°C.

E-1.4 Base-Exchange Silicate Absorption Tube — consists of reservoir of approximately 50 mm long and 25 mm diameter, absorption tube of 5 to 6 mm in diameter and approximately 140 mm long, ending in capillary tube of 10 mm and of such bore that when charged, the rate of flow is not more than 1 ml/min. The apparatus is made up of glass chromatographic tubes fused together into a continuous unit. For preparing the apparatus for use, place over the upper end of the capillary, with the aid of a glass rod, pledget of fine glass wool and add to the absorption tube water-suspension of 1 to 2 g of purified base-exchange silicate (*see* **E-2.8**) taking care to wash down all the silicate from the walls of the reservoir. Prevent the tube from draining by placing a rubber cap, over lower end of the capillary, filled with water.

E-1.5 Glass-Stoppered Cylinders or Centrifuge Separating Funnels — of 25 ml capacity.

E-2. REAGENTS

E-2.1 Sodium Hydroxide Solution — 15 percent (*w/v*).

E-2.2 Potassium Ferricyanide Solution — 1 percent (*w/v*). If stored in stoppered brown bottle in cool, dark place, this reagent is stable.

E-2.3 Standard Sulphuric Acid — 0·1 N.

E-2.4 Sodium Acetate Solution — 2·5 M. Dissolve 205 g of anhydrous sodium acetate or 345 g of sodium acetate trihydrate in water and dilute to one litre.

E-2.5 Enzyme Solution — Prepare fresh daily, using 2 percent solution or suspension of enzyme potent in diastatic and phosphorolytic activity in 2·5 M sodium acetate solution (*see* **E-2.4**).

NOTE — The enzyme potent in diastatic and phosphorolytic activity, available are 'Polidase-S', 'Mylase-P', 'Clarase' and 'Taka-Diastase'.

E-2.6 *Iso*butanol — giving blank reading of 1·5 galvanometer scale division or less. If necessary, distil the commercial product in all-glass apparatus or shake with activated charcoal to remove fluorescent impurities.

E-2.7 Potassium Chloride Solution — 25 percent (*w/v*).

E-2.8 Purified Base-Exchange Silicate — Place convenient quantity of base-exchange silicate of particle size completely passing through 300-micron IS Sieve, but completely retained in 180-micron IS Sieve in a Buchner funnel. Wash it with 3 percent solution of hot acetic acid, maintaining the acid in contact with the material for 10 to 15 minutes. Drain off the acid by vacuum and then wash it with approximately 250 ml of hot 25 percent potassium chloride solution (*see* **E-2.7**), allowing to remain for 10 to 15 minutes before draining by vacuum. Repeat the acid wash. Then wash with several portions of hot water until the extract is free from chloride, as shown by silver nitrate test. Dry the base-exchange silicate at room temperature or in an oven below 100°C and store in stoppered bottle.

E-2.9 Stock Thiamine Solution — Dry standard thiamine hydrochloride over phosphorus pentoxide in a desiccator at least for 24 hours. Dissolve 100 mg in 25 percent ethyl alcohol and dilute to one litre with 25 percent ethyl alcohol. This solution is stable for several months if kept in refrigeration.

E-2.10 Standard Thiamine Solution — Dilute 5 ml of the stock thiamine solution (warmed to room temperature) to 100 ml with water. Dilute 4 ml of this intermediate concentration to 100 ml with 0·1 N sulphuric acid (5 ml of this standard solution contains one microgram of thiamine and hence the difference between the total and the blank readings of the galvanometer in the case of standard solution would correspond to 1 microgram of thiamine). Prepare fresh daily.

E-3. PROCEDURE

E-3.1 Extraction — Grind the material so that it passes through an 850-micron IS Sieve. Weigh accurately about 1 g of the powdered material and transfer it to a 100-ml flask. Add 50 ml of 0·1 N sulphuric acid and heat over the water-bath maintained at 100° ± 1°C for 10 minutes. Cool the flask to 40°C or below and add 5 ml of enzyme solution (*see* **E-2.5**). Incubate at least for 4 hours at 37° to 40°C. Cool and dilute to 100 ml. Stir the digested extract thoroughly and filter through Whatman No. 41 or its equivalent filter paper discarding a few millilitres of the filtrate. If the filtrate is turbid or if filtration is extremely slow, centrifuge the suspension prior to filtration.

E-3.2 Purification — It is necessary to use this step only on highly coloured extracts or extracts with interfering substances. To determine, if

interfering substances are present, test duplicate samples with and without this step. Add a 10-ml aliquot of the clarified filtrate to the absorption tube while base-exchange silicate is still wet. Wash the reservoir and the column with three successive 5-ml portions of hot water to ensure proper distribution of the thiamine on absorption tube. Collect the purified absorbed thiamine from the base-exchange silicate by filling the tube with 15 ml of boiling potassium chloride solution. This is collected in a 25-ml glass-stoppered graduated flask to permit volume to be measured completely.

E-3.3 Oxidation — Mix the purified extract well by inverting three or four times, and pipette 5 ml into a 25-ml glass-stoppered centrifuge separating funnel. Pipette a similar 5-ml aliquot into a second glass-stoppered centrifuge separating funnel to be used for blank. Number them as 1 and 2 respectively. To the first funnel add 3 ml of alkaline ferricyanide solution. (Prepare this alkaline potassium ferricyanide fresh daily by diluting 3 ml of one percent potassium ferricyanide to 100 ml with 15 percent sodium hydroxide solution. If preferred, add 3 ml of alkali and one drop of potassium ferricyanide separately, obviating daily preparation of the mixture.) To the second vessel add 3 ml of 15 percent sodium hydroxide solution. Mix these gently taking 30 seconds and add 15 ml of *iso*butanol to each. Shake vigorously for 60 seconds. Centrifuge for 60 seconds at a rate of 1 800 rev/min to separate the layers. Draw off the aqueous layer, and add 1 ml of 95 percent ethyl alcohol to the *iso*butanol layer and mix. Decant 10 ml of *iso*butanol into the cuvet for reading the fluorescence of thiochrome.

E-3.4 Measurement — Determine the fluorescence on 10 ml of the *iso*butanol solution in terms of galvanometer deflections, operating the fluorometer for blank and sample solutions.

NOTE — Take the fluorometer reading as quickly as possible to minimize destruction of thiochrome by inciting light. It is advisable to conduct measurements in room with subdued light.

E-3.4.1 Standardize the fluorometer with either quinine sulphate solution or a glass standard. Periodic checks with quinine sulphate solution or glass standard during the day are desirable to keep the instrument standardized. If initial reading is not given, readjust. Calibrate the galvanometer in terms of deflection with standard thiamine solution. With each set of samples treat two samples of standard thiamine solution exactly as the unknown and determine the fluorescence for blank and standard solution.

E-4. CALCULATION

E-4.1 Thiamine, milligrams in 100 g of the material $= \frac{R_x - R_{xb}}{R_s - R_{sb}} \times \frac{E}{5} \times \frac{V}{Z} \times \frac{1}{S} \times 0{\cdot}1$

where

R_x = fluorometer reading with the sample,
R_{xb} = blank fluorometer reading with the sample,
R_s = fluorometer reading with the standard,
R_{sb} = blank fluorometer reading with the standard,
E = volume in ml of purified extract collected,
V = volume in ml of extraction,
Z = volume in ml of extract passed through absorption tube, and
S = weight in g of the sample.

APPENDIX F

[*Table* 1, *Item* (vi)]

DETERMINATION OF RIBOFLAVIN

F-1. APPARATUS

F-1.1 Autoclave or Water-Bath

F-1.2 Photo-electric Fluorometer — Prepare the instrument according to the manufacturer's directions.

F-1.3 Glassware — Amber-colour glassware shall be used wherever practicable. Exposure to light shall be reduced to the minimum throughout the experiment.

F-2. REAGENTS

F-2.1 Dilute Sulphuric Acid — 0·1 N.

F-2.2 Sodium Acetate Solution — 2·5 M. Dissolve 340 g of sodium acetate trihydrate and dilute to one litre.

F-2.3 Potassium Permanganate Solution — 4 percent (*w*/*v*). Prepare fresh daily.

F-2.4 Hydrogen Peroxide — 3 percent. Dilute 30 percent hydrogen peroxide 1 : 10 with water. Assay immediately before use and discard if the assay is less than 2·5 percent.

F-2.5 Riboflavin Standard

F-2.5.1 Dry standard riboflavin over phosphorus pentoxide in a desiccator for 24 hours. Dissolve 50 mg in 1 500 ml of water and 2·4 ml glacial acetic

acid in a 2-litre flask. Warm to aid solution. Cool and make up to volume. Store under toluene in amber bottle and refrigerate.

F-2.5.2 Dilute 20 ml of riboflavin solution (**F-2.5.1**) to 50 ml with water.

F-2.5.3 Prepare working solution by diluting 10 ml of riboflavin solution (**F-2.5.2**) with water to 100 ml. 1 ml of this solution contains one microgram of riboflavin. Prepare fresh daily. Protect from light.

F-2.6 Sodium Hydrosulphite — pure, powdered and protected from light.

F-3. PROCEDURE

F-3.1 Extraction — Grind the material to pass through an 850-micron IS Sieve. Mix the material well. Weigh about 2 g of the material accurately and transfer to a 100-ml flask. Add 75 ml of dilute sulphuric acid and either autoclave at 1·2 kg/cm² of pressure for 30 minutes or immerse the flask in boiling water-bath for 30 minutes, shaking the flask every 5 minutes. Let it cool to room temperature.

F-3.2 Adjustment of *p*H — Add 5 ml of sodium acetate solution and mix well. Let stand at least for one hour. At this stage the *p*H of the solution is approximately 4·5. Make up the volume and filter through medium fast paper, such as Whatman No. 2 or No. 4. The filter paper may be tested for riboflavin absorption by comparing the galvanometer readings of filtered and unfiltered standard riboflavin working solution. Collect the filtrate in a 100-ml graduated glass-stoppered flask to enable to measure the volume of the solution accurately.

F-3.3 Oxidation of Impurities — In two test-tubes, each of 2·5 cm in diameter and with stirring rods marked A and B, carry out oxidation as follows:

	Tube A Blank	*Tube B Material*
Sample solution (ml)	10	10
Standard solution (ml)	1	—
Water (ml)	—	1
Potassium permanganate, 4 percent solution (ml)	1	1
Time lapse (minutes)	4	4
Hydrogen peroxide, 3 percent (ml)	1	1

Stir after the addition of potassium permanganate. Shake after adding peroxide until foaming is negligible.

F-4. MEASUREMENTS

F-4.1 Adjust the fluorometer so that the glass standard or the sodium fluorescence solution gives suitable galvanometer deflection as directed

for the instrument. Determine the fluorescence of solutions A and B (from the test-tubes so marked). Make readings with no more than 10 seconds exposure.

F-4.2 To solution B in the cuvet add 20 mg of sodium hydrosulphite, stir and determine blank fluorescence C. Do not use reading C after colloidal sulphur begins to form.

NOTE 1 — Avoid excess of hydrosulphite. It is necessary to take readings rapidly, before colloidal sulphur begins to form. Colloidal sulphur will raise apparent fluorescence. An excess of hydrosulphite may change fluorescent properties of blank, owing to high salt concentration. Hydrosulphite may be added while the cuvet is in the instrument. Stir with ultra-violet light on; watch destruction of fluorescence. Cover the cuvet chamber and switch on the galvanometer immediately, even if some hydrosulphite remains undissolved. Undissolved salts settle out rapidly and do not interfere.

NOTE 2 — When several samples are to be analyzed, it may be more convenient to use a solution of hydrogen sulphite. Dissolve 2 g of sodium bicarbonate in 100 ml of water. Cool in ice-bath. When thoroughly cold, dissolve 5 g of sodium hydrosulphite. When kept in ice-bath this solution is stable for about 3 hours. Use 0·5 ml of this solution. Correction shall be made for volume change when this solution is used.

F-5. CALCULATION

F-5.1 Riboflavin, milligram in 100 g of the material $= \frac{B-C}{A-B} \times \frac{R_1}{V_1} \times \frac{V}{W} \times 0{\cdot}1$

where

B = fluorometer reading of the sample plus water,

C = fluorometer reading after the addition of sodium hydrosulphite,

A = fluorometer reading of the sample plus riboflavin standard,

R_1 = microgram of standard riboflavin added to tube marked A (**F-3.3**),

V_1 = volume in ml of sample solution taken for measurement,

V = volume in ml of final sample solution (**F-3.2**), and

W = weight in g of the sample (**F-3.1**).

APPENDIX G

[*Table* 1, *Item* (vii)]

DETERMINATION OF NICOTINIC ACID

G-1. APPARATUS

G-1.1 Photo-electric Colorimeter — with 400 to 420 mμm filter or wave band.

G-1.2 Centrifuge — 2 000 rev/min.

G-1.3 Water-Bath — maintained at 100°C.

G-2. REAGENTS

G-2.1 Concentrated Hydrochloric Acid

G-2.2 Sulphuric Acid — approximately 10 N.

G-2.3 Sulphuric Acid — approximately 2 N.

G-2.4 Sulphuric Acid — approximately 0·2 N.

G-2.5 Sodium Hydroxide Solution — approximately 10 N.

G-2.6 Sodium Hydroxide Solution — approximately 0·5 N.

G-2.7 Lloyd Reagent (Hydrated Aluminium Silicate)

G-2.8 Lead Nitrate — pulverized.

G-2.9 Phenolphthalein Solution — 1 percent in 70 percent ethyl alcohol.

G-2.10 Potassium Phosphate — tribasic, crystals.

G-2.11 Phosphoric Acid Solution — 20 percent by weight.

G-2.12 Indicator Paper — for *p*H 4·5.

G-2.13 Potassium Phosphate Solution — monobasic, 10 percent by weight.

G-2.14 Standard Nicotinic Acid Stock Solution — Dissolve 500 mg of standard nicotinic acid, previously dried and stored in a desiccator over phosphorus pentoxide, in 5 ml of 10 N sulphuric acid in a 500-ml volumetric flask. Dilute to volume with water. If kept under refrigeration and protected from light, it is stable for one year. Each millilitre of this solution contains 1 mg of nicotinic acid.

G-2.15 Standard Nicotinic Acid Working Solution — Dilute 5 ml of the stock solution (**G-2.14**) to 200 ml with water. This solution contains 25 μg of nicotinic acid per millilitre. Prepare fresh daily.

G-2.16 Cyanogen Bromide Solution — 0·5 N (CAUTION: Cyanogen bromide is highly poisonous. Prepare and use only in hood. Do not breathe any vapours. If the solution comes in contact with skin, wash with water at once). Dissolve 53 g of cyanogen bromide crystals in water in a 1 000-ml volumetric flask. Dilute to mark.

OR

Quickly weigh 80 g of cold bromine (use hood) by pouring about 27 ml into a tared cold graduated cylinder. Transfer bromine immediately to a

1 000-ml glass-stoppered volumetric flask containing about 500 ml of cold water. Cool in ice-bath. Dissolve 50 g of sodium cyanide in water in a 500-ml volumetric flask. Dilute to volume with water. Add sodium cyanide solution to bromine water slowly in small amounts from a burette with constant shaking, until the solution just becomes colourless. Add 10 drops of sodium cyanide in excess. Dilute to volume with water. If stored in a glass-stoppered brown bottle, the solution may be used for several months.

G-2.17 Hydrochloric Acid — approximately 8 N.

G-2.18 Hydrochloric Acid — approximately 0·5 N.

G-2.19 *p*-Methylaminophenol Sulphate Solution — 5 percent by weight. Dissolve 10 g of the crystals in 200 ml of 0·5 N hydrochloric acid in an amber bottle. Prepare fresh daily, immediately before use.

G-3. PROCEDURE

G-3.1 Weigh accurately about 0·5 g (estimated to contain 100 μg of nicotinic acid) of the material and transfer to a centrifuge tube. Dilute to 15 ml with water. From a burette add 5 ml of concentrated hydrochloric acid. Place the centrifuge tube in the rack in a boiling water-bath and let remain for one hour, stirring with a glass rod occasionally. Remove from the bath and cool to room temperature by placing it in a cold water-bath. Rinse the stirring rod with a small amount of water, dilute the solution to 25 ml with water and stir well. Filter this solution into a small beaker, using Whatman No. 40 filter paper or its equivalent known not to absorb nicotinic acid.

G-3.2 Pipette out a 10-ml aliquot into a 50-ml beaker. Add approximately 2 ml of 10 N sodium hydroxide solution, cool, adjust *p*H to 0·5 to 1·0 by adding 10 N sodium hydroxide solution or concentrated hydrochloric acid dropwise.

G-3.3 Transfer the aliquot quantitatively to the centrifuge tube containing 2 g of Lloyd reagent, using a small amount of 0·2 N sulphuric acid as wash solution. Stir for a minute, wash the stirring rod and the sides of the tube with 0·2 N sulphuric acid and centrifuge for 5 minutes at 2 000 rev/min. Discard the supernatant liquid. Wash the residue with 10 ml of 0·2 N sulphuric acid, stirring sufficiently to break up clumps of Lloyd reagent. Wash the stirring rod and sides of the tube with 0·2 N sulphuric acid and centrifuge as before. Discard the supernatant liquid, draining completely. Add 15 ml of 0·5 N sodium hydroxide solution, stir for one minute after breaking up clumps. Wash the stirring rod with water. Dilute to 21·2 ml with water. Stir to mix thoroughly. Centrifuge as before. Drain the supernatant liquid completely into centrifuging tube containing one gram of pulverized lead nitrate and one drop of phenolphthalein solution. Stir until pink colour disappears. Centrifuge as before. Decant the liquid

into a centrifuging tube containing one drop of phenolphthalein. Add just enough potassium phosphate crystals to give pink colour. Carefully add phosphoric acid solution to change *p*H to 4·5, testing with a *p*H indicator paper. Centrifuge. Decant the supernatant liquid completely into a glass-stoppered graduated flask to enable the volume measurement of this sample extract.

G-3.4 Set up in a rack one test tube for instrument blank, one for standard nicotinic acid and one for sample and follow the direction for adding reagents as given below:

	Instrument Blank Tube	*Standard Working Nicotinic Acid Tube*	*Sample Tube*
Sample extract (ml)	—	—	4
Standard nicotinic acid working solution (ml)	—	1	—
Water (ml)	7	6	3
Potassium phosphate (monobasic) solution (ml)	1	1	1
Cyanogen bromide solution (ml)	2	2	2
p-Methylaminophenol sulphate solution (ml)	10	10	10

First transfer 4-ml aliquots of sample extract to sample tube. Add one ml of standard nicotinic acid working solution to standard tube. To all tubes add one ml of potassium phosphate solution and specified quantity of water. Place all the tubes in a water-bath at 70°C for 5 minutes. Under diffused light in hood add 2 ml of cyanogen bromide solution from a burette to all the tubes at 15 seconds intervals. Mix by swirling. Five minutes after adding cyanogen bromide solution to the first tube, transfer it to water-bath at 25°C. Transfer the other tubes, in order, at 15 seconds intervals. Add 10 ml of *p*-methylaminophenol solution to all the tubes and mix the contents well. Place the tubes in a dark place for one hour. Wipe off the tubes with lint-free cloth before making colour readings.

G-4. MEASUREMENTS

G-4.1 Using a 400- to 420-mμm filter, read the absorbance with the photoelectric colorimeter. First set the instrument to read 100 with the instrument blank tube. Then take the reading for the sample tube. Take the reading directly for standard tube. Convert the galvanometer reading of the sample tube and the standard nicotinic acid tube to absorbance by subtracting the value of the logarithm to the base 10 of the respective galvanometric reading from 2.

G-5. CALCULATION

G-5.1 Nicotinic acid, milligram in 100 g of the material $= \frac{25\,B}{A} \times \frac{V}{4} \times \frac{25}{10} \times \frac{1}{W} \times 0{\cdot}1$

where

B = absorbance of the sample,
A = absorbance of the standard nicotinic acid,
V = volume in ml of the sample extract (**G-3.3**), and
W = weight in g of the sample.

APPENDIX H

[*Table* 1, *Item* (viii)]

DETERMINATION OF PANTOTHENIC ACID

H-1. APPARATUS

H-1.1 Photo-electric Colorimeter

H-1.2 Centrifuge

H-1.3 Mechanical Shaker — in 30°C thermostat.

H-1.4 Autoclave

H-1.5 Sterilizer — steam.

H-1.6 Glass Electrodes

H-2. REAGENTS

H-2.1 Yeast — *Saccharomyces carlsbergensis* (Fleischmann Culture 4228).

H-2.2 Malt Agar — Difco or its equivalent.

H-2.3 Sterile Saline Solution — 0·9 percent (*w/v*). Dissolve 9 g of sodium chloride and dilute to one litre, sterilize and cool.

H-2.4 Moist Baker's Yeast

H-2.5 Sugar and Salt Solution — Dissolve 200 g of anhydrous glucose, 2·2 g of potassium hydrogen phosphate (KH_2PO_4), 1·7 g of potassium

chloride, 0·5 g of calcium chloride crystals ($CaCl_2$, $2H_2O$), 0·5 g of magnesium sulphate crystals ($MgSO_4$, $7H_2O$), 0·01 g of ferric chloride crystals ($FeCl_3$, $6H_2O$) and 0·01 g of manganese sulphate crystals ($MnSO_4$, $4\ H_2O$) in water and dilute to one litre.

H-2.6 Potassium Citrate Buffer — Dissolve 100 g of potassium citrate monohydrate and 20 g of citric acid monohydrate in water and dilute to one litre.

H-2.7 Thiamine Hydrochloride Solution — 10μ g/ml.

H-2.8 Pyridoxine Solution — 10 μg/ml.

H-2.9 Inositol Solution — 1 mg/ml.

H-2.10 Biotin Solution — 0·8 μg/ml.

H-2.11 Ammonium Sulphate Solution — 150 mg/ml.

H-2.12 Asparagine Solution — Dissolve 3 g of *l*-asparagine in 100 ml of water by boiling. Cool and dilute to 100 ml. Sterilize the solutions in **H-2.5** to **H-2.12** by heating in flowing steam for 30 minutes on three consecutive days and then store at room temperature until used.

H-2.13 Standard Pantothenate Solution — Make a solution of synthetic *d*-calcium pantothenate containing 1 mg/ml; store in refrigerator not longer than 3 weeks. Immediately before using, dilute a portion of this stock solution to give working standard solution containing 0·1 μg/ml.

H-2.14 Enzyme Solution — Weigh 20 g of the enzyme potent in diastatic and phosphorolytic activity into 20 ml water, stir, make into a paste and dilute to 200 ml. Centrifuge to remove any insoluble matter and filter the supernatant fluid through sterile serum No. 1 filter paper or its equivalent into a sterile receiving flask. Store under benzene in refrigerator.

NOTE — The enzymes potent in diastatic and phosphorolytic activities available are 'Poledase-S', 'Mylase-P', 'Clarase' and 'Taka-Diastase'.

H-2.15 Basal Medium — Mix the following:

Sugar and salt solution (**H-2.5**)	100 ml
Potassium citrate buffer (**H-2.6**)	20 ml
Inositol solution (**H-2.9**)	10 ml
Ammonium sulphate solution (**H-2.11**)	10 ml
Thiamine hydrochloride solution (**H-2.7**)	10 ml
Pyridoxine solution (**H-2.8**)	10 ml
Biotin solution (**H-2.10**)	10 ml
Asparagine solution (**H-2.12**)	25 ml

Dilute to 200 ml. Large batches of basal medium may be prepared and preserved indefinitely in frozen state.

H-3. PROCEDURE

H-3.1 Preparation of Yeast Inoculum — Prepare fresh slant of culture (**H-2.1**) on Difco malt agar or its equivalent and incubate for 24 hours at 30°C. These agar slants are not to be stored in refrigerators for more than a month. One day preceding to the determination, make fresh transfer and incubate at 30°C. Remove a quantity of the fresh growth with sterile wire loop and suspend in 10 ml of sterile saline solution in the colorimeter tube. Using photo-electric colorimeter, previously calibrated against moist yeast, adjust concentration to the equivalent of 3 mg/ml, by adding yeast or saline solution as required. Dilute 5 ml of adjusted suspension with 45 ml of saline solution contained in sterile Erlenmeyer flask.

H-3.2 Preparation of Sample, Enzyme Digestion and Aqueous Extraction — Weigh the amount of sample containing approximately 10 to 20 μg of the pantothenate into 40-ml test-tube graduated at 10 and 20 ml. Add one millilitre potassium citrate buffer solution and water to make the volume to 10 ml. Heat in flowing steam for 5 minutes, cool and add an amount of enzyme solution so that the weight of enzyme solution is equal to a sample weight. Make up the volume to 20 ml, mark with water and add 0·5 ml of benzene to form a layer. Cork tightly. Incubate at 45°C for 2 days or at 37·5°C for 3 days. Transfer the contents of the tube to a 200-ml beaker with approximately 130 ml of water and adjust *p*H to 5·6 to 5·7. Autoclave for 15 minutes at 7·5 kg/cm^2. Cool and dilute to 200 ml. If necessary, centrifuge to clear the final solution.

H-3.3 Determination of Free Pantothenic Acid — Weigh an amount of the sample containing approximately 10 to 20 μg of pantothenate. Add 1 ml of potassium citrate buffer solution and 150 ml of water. Autoclave for 15 minutes at 7·5 kg/cm^2. Cool and dilute to 200 ml. Centrifuge. In case the autoclaved solution is not clear after centrifuging, add 25 ml of the enzyme solution and incubate at 45°C for 30 minutes. Now, on centrifuging clear supernatant should result.

H-3.4 Microbiological Assay — Place 5 ml of basal pantothenate-free medium in each of a series of 16 Pyrex test-tubes. Into successive tubes pipette 1, 2, 3, and 4 ml of sample extract and sufficient water to give total volume of 9 ml, including medium. With each assay series group include a reference series containing 0, 50, 100, 150, 200, 300 and 400 μg of pantothenate and again adjust volume in each tube to 9 ml. Plug all tubes with cotton, steam for 10 minutes and cool. Under aseptic condition, introduce into each tube 1 ml of yeast inoculum. Place the tubes in mechanical shaker for 16 hours at 30°C. After incubation, measure yeast growth with photo-electric colorimeter, using 660-mμm filter. Estimate pantothenate content of each assay tube by interpolation on standard curve constructed from results obtained with known quantities of pantothenate. Replace on mechanical shakers and two hours later, repeat yeast growth

estimation for both known and unknown sample tubes. Average all values agreeing within ±10 percent of the mean and report as calcium pantothenate.

NOTE — If more than two of eight assay tubes for any sample deviate from average by more than ± 10 percent, it is advisable to repeat the assay.

APPENDIX J

[*Table* 1, *Item* (ix)]

TEST FOR VIABILITY

J-1. REAGENT

J-1.1 Yeast Nutrient Mixture — Grind and mix thoroughly 4 g of sucrose, 0·50 g of di-ammonium phosphate and 0·25 g of magnesium sulphate.

J-2. PROCEDURE

J-2.1 Add 4·5 g of yeast nutrient mixture to a 100-ml pasteur flask containing 50 ml of tap water and sterilize by heating in an autoclave for 20 minutes at 115°C; add with aseptic precautions 2 g of sample and incubate at 30°C. Collect the gas evolved in a graduated tube inverted over the outlet tube in a trough of water and measure the volume of gas at the end of 6 hours. Repeat the operation omitting the sample, and subtract the volume of gas evolved from that obtained in the first determination. The sample shall be considered to have passed the test if the difference is not greater than 10 ml.

APPENDIX K

(*Clause* 4.1)

SAMPLING OF FODDER YEAST

K-1. GENERAL REQUIREMENTS OF SAMPLING

K-1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed.

K-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

K-1.2 The sampling instrument shall be clean and dry when used.

K-1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers being sampled from adventitious contamination.

K-1.4 The samples shall be placed in clean, odourless and dry glass containers almost completely filled by the sample.

K-1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

K-1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

K-1.7 Sampling shall be done by a person agreed to between the purchaser and the vendor and in the presence of the purchaser (or his representative) and the vendor (or his representative).

K-2. SCALE OF SAMPLING

K-2.1 Lot — All the containers in a consignment belonging to the same batch of manufacture shall constitute a lot.

K-2.1.1 Samples shall be tested from each lot for ascertaining conformity of the material to the requirements of this specification.

K-2.2 The number of containers to be tested from a lot shall depend on the size of the lot and shall be in accordance with Table 2.

TABLE 2 NUMBER OF CONTAINERS TO BE SELECTED FOR SAMPLING

Total Number of Containers in the Lot N	Number of Containers to be Selected n
(1)	(2)
3 to 50	3
51 „ 200	4
201 „ 400	5
401 „ 650	6
651 and over	7

K-2.3 These containers shall be selected at random from the lot. To ensure the randomness of selection, a random number table as agreed to

between the purchaser and the supplier shall be used. In case such a table is not available, the following procedure shall be adopted:

Starting from any container, count them as 1, 2, 3, ..., etc, up to r and so on, in one order. Every rth container thus counted shall be chosen so as to give the required number of containers in the sample, r being the integral part of N/n.

K-3. TEST SAMPLES AND REFEREE SAMPLES

K-3.1 Preparation of Individual Samples — Empty out the contents of the container on a sheet of paper and mix thoroughly. Cone and quarter as often as necessary till about 500 g of the material are left. From this take about 150 g of the material and divide into three equal parts. Each part so obtained shall constitute an individual sample representing the container and shall be transferred immediately to thoroughly clean and dry containers, sealed air-tight and labelled with the particulars given under **K-1.5**. The individual samples so obtained shall be divided into three sets in such a way that each set has a sample representing each selected container. One of these sets shall be marked for the purchaser, another for the vendor and the third for the referee.

K-3.2 Preparation of a Composite Sample — From the material from each selected container remaining after the individual sample has been taken, equal quantities of the material shall be taken and mixed together so as to form a composite sample weighing about 300 g. This composite sample shall be divided into three equal parts and transferred to clean and dry containers made of glass and labelled with the particulars given in **K-1.5**. One of these composite samples shall be for the purchaser, another for the vendor and the third for the referee.

K-3.3 Referee Samples — Referee samples shall consist of a set of individual samples (**K-3.1**) and one composite sample (**K-3.2**) marked for this purpose and shall bear the seals of the purchaser and the vendor. These shall be kept at a place agreed to between the two.

K-4. NUMBER OF TESTS

K-4.1 Tests for description given under **2.1** and crude protein content shall be conducted on each of the samples constituting a set of individual test samples (**K-3.1**).

K-4.2 Tests for the remaining characteristics, namely, moisture, total ash, crude fibre, thiamine, riboflavin, nicotinic acid, pantothenic acid, and viability, shall be conducted on the composite samples as prepared under **K-3.2**.

K-5. CRITERIA FOR CONFORMITY

K-5.1 The lot shall be considered satisfactory in respect of the requirements tested in **K-4.1**, if each of the individual sample satisfies all these requirements.

K-5.2 The lot shall be considered satisfactory in respect of the requirements tested in **K-4.2**, if the test results on the composite sample satisfy the corresponding requirement.

K-5.3 The lot shall be declared to be in conformity with all the requirements of this specification, if it has been found satisfactory in **K-5.1** and **K-5.2.**

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